

Please replace page 34 with substitute pages 34 and 34a.

Please replace the paragraph spanning lines 3-16 on page 35 with the following paragraph: -

3-3 Confirmation on the processing of DNA incorporated into cells

B²
To confirm as to how far the cloned DNA fragments of 50-200 bp incorporated into cell by processing are further processed within cells and how the base sequences of the DNA fragments influence on the cells, a PCR amplification was performed. The PCR reactive solution contained 0.2mM dNTP, 10 pmole of primer, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, and 2.5 units of *Taq* polymerase. 5'-CTCCCGGCCCGCCATG-3' (SEQ ID NO: 5) and 5'-TTGGGAGCTCTCC-3' (SEQ ID NO: 6) (Table I) were synthesized and used as a PCR primer. The PCR reaction was repeated 35 times under the following condition: denaturation (at 94°C for 30 sec.); primer annealing (at 42°C for 30 sec.); primer extension (at 72°C for 50 sec.). The PCR product was run on 8% native-PAGE in a TBE buffer solution and was stained with ethidium bromide. Subsequently, DNA was separated from the gel of the PCR reaction product. The separated DNA was precipitated by ethanol, and was dissolved in PBS for cell culture or was dissolved in TE buffer solution for the detection of the activity of the endonuclease.

REMARKS

The foregoing amendments and these remarks are in response to the Notice to Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures.

The specification has been amended in the patent application and a "Sequence Listing" has been provided. The affected specification pages are shown in a section entitled Marked-Up Version To Show Changes using standard underlining and bracketing format to highlight the changes made. Applicants provide a computer readable form (CRF) copy of the "Sequence Listing", an initial paper or compact disc copy of the "Sequence Listing", as well as this Amendment directing its entry into the application. Applicants also provide a Statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc)


sequence listing and, where applicable, includes no new matter, as required by 37 C.F.R. 1.821(f).

Applicants submit that no new matter is entered by this Preliminary Amendment and respectfully request entry of the amendment and examination on the merits.

A separate clean set of the amended pages is attached.

Respectfully submitted,

Date: 3/28/02


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Docket No. 9250-2

Table I.

	oligonucleotides	using
A	TTAAACGTTTCAC(SEQ ID NO:1)	CpG motif
5 B	AAGTGAACGTTTT(SEQ ID NO:2)	CpG motif
C	AGCAGCGCTAA(SEQ ID NO:3)	CpG motif
D	AATTAGCGCTG(SEQ ID NO:4)	CpG motif
E	CTCCCGGCCGCCATG(SEQ ID NO:5)	PCR primer
F	TTGGGAGCTCTCCC(SEQ ID NO:6)	PCR primer
10 G	GTTCCTCCAGTCACGAC(SEQ ID NO:7)	sequencing (pUC/M13 forward)
H	CAGGAAACAGCTATGAC(SEQ ID NO:8)	sequencing (pUC/M13 reverse)

The test results show that when 25 ug/ml of bacterial DNA was incubated with IM9 cell, the DNA was properly processed in the cell culture and incorporated into the cells. This was confirmed by southern hybridization (Fig. 20). The presence of 100-200 bp DNA was found 30 minutes after the cell culture. The amount of 100-200 bp DNA in the cells was decreased with the lapse of the culture time. The results suggest that properly processed DNA was incorporated into IM9 cell line and the processing was continued in cells.

To identify the property of the product obtained by the enzyme activity of the endonuclease, the bacterial DNA incorporated into cells was isolated and its DNA sequence was analyzed as shown in Fig. 21. As shown in Table 2, the reaction product of the endonuclease had a characteristic base sequence, i.e., CpG motif carrying two purine bases at 5' end and two pyrimidine bases at 3' end. It is known that CpG motif activates B cell or macrophage in an immune system and promotes the secretion of cytosine and IgM and is present in bacterial DNA at high frequency. Accordingly, it was now found that the endonuclease present in IM9 cell culture solution and the DNA processing by the enzyme activity in the cell generate CpG motif which functions to activate immune cells.

Table 2. 50-200 bp DNA fragments produced by endonuclease reaction in cell

	Sequence	Precursor
EC1AGAGCAGCGCTAATGTCTATCGATGATT.... (SEQ ID NO: 9)GTCAAAACGTTACCA..... (SEQ ID NO: 10)	<i>E. coli</i> from bases 2874223 to 2885334 of the complete genome (5416-5614)
EC2TTAACAAACGTTGGGGCGATTGAGAGCGAT GGCGTTGATTTCATGTAACGAAGCTAACGTG GTGAACAAACGATGATGGCGCACCGAGAAAT.....	<i>E. coli</i> from bases 4067762-4083201 of the complete genome (4741-4852)
EC3CCCATGACCGCACCGCA.....ATTCCATCGCCATCTCAAACTTCGGTAA	<i>E. coli</i> from bases 2244905 to 2255428 of the complete genome (2027-2108)
EC4	TGCCTCGGAGTTACCTAATTCCATCGCCA TCTCAAACTTCGGTAAA	<i>E. coli</i> from bases 2244905 to 2255428 of the complete genome (2064-2109)
EC5CCTTTGACGTTGAGTCCACGTTCTTA...CCTATCTCGGTCTATT....	<i>E. coli</i> plasmid synthetic cloning vector pET31F (268 376)
EC6	TTACGGTTCCTG.....TTTCCTGGCGTTATCCC...	Plasmid pKF3 from <i>E. coli</i> (2004-2066)
EC7	GTGACCATATGGGAGAGCTCC..... ACGCGTTGGATG..... AGCTTGGCGTAATCAT	Cloning vector pGEM-5Zf(-) (75-170)



	Sequence	Precursor
EC8TTTCCTGGCGTTATCCC..... ...GCTGATACGCTCGCCGCAGCCGAACG ACCGAGCGCAGCGAGTACAGTGAAGCGAGG AAGCG	(SEQ ID NO: 22) (SEQ ID NO: 23) Sequence from patent US 4921698
PD1	CTTCCTGGCTCACTGACTCGCTGGCGCTCG GTGGTTGGGCTGCGGCGACGTTATCAG.....	(SEQ ID NO: 24) pGEM-T vector (401-519)
PD2GGTCTGACGCTCAGTGAACGA AAACTCAGCGTTAAGGG.....	(SEQ ID NO: 25) pGEM-T vector (1130-1306)
PD3TAAAGAACGTGGACTCCAA CGTCAAAAGGGCGGAAAAACCGTCTAT CAGGGCGATGCCCC.....	(SEQ ID NO: 26) pGEM-T vector (2513-2610)
PD4TTCCCAACGATCAAGGCGAGTTACA..... CTCGATCGTTGTACG.....	(SEQ ID NO: 27) (SEQ ID NO: 28) pGEM-T vector (1685-1795)
EC9TGCTGTTGGGCACCAACAATCAGCGCC GACTTTAA.....	(SEQ ID NO: 29) <i>E. coli</i> from bases 220025 to 231029 of the complete genome (829-872)
HC1TTCGATTCGAT.....	(SEQ ID NO: 30) <i>Homo sapiens</i> satellite 2 repetitive element DNA

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3-3 Confirmation on the processing of DNA incorporated into cells

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Killer

To confirm as to how far the cloned DNA fragments of 50-200 bp incorporated into cell by processing are further processed within cells and how the base sequences of the DNA fragments influence on the cells, a PCR amplification was performed. The PCR reactive solution contained 0.2 mM dNTP, 10 pmole of primer, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, and 2.5 units of *Taq* polymerase. 5'-CTCCCGGCCGCCATG-3' (SEQ ID NO: 5) and 5'-TTGGGAGCTCTCC-3' (SEQ ID NO: 6) (Table I) were synthesized and used as a PCR primer. The PCR reaction was repeated 35 times under the following condition: denaturation (at 94°C for 30 sec.); primer annealing (at 42°C for 30 sec.); primer extension (at 72°C for 50 sec.). The PCR product was run on 8% native-PAGE in a TBE buffer solution and was stained with ethidium bromide. Subsequently, DNA was separated from the gel of the PCR reaction product. The separated DNA was precipitated by ethanol, and was dissolved in PBS for cell culture or was dissolved in TE buffer solution for the detection of the activity of the endonuclease.

To confirm as to which form of the end product exists after the product obtained by the PCR amplification in a vector cloning 100-200 bp of bacteria DNA, which was cut by the activity of the endonuclease, is introduced into cells, the EC1, EC2, and HC1 DNA fragments were labeled with ³²P by a random-priming method using random primer and Klenow enzyme. 100 ng/ml of the DNA fragments labeled with ³²P were incorporated into IG9 cell line, U937 cell line, and U937 cell line treated with TPA. Whether DNA was incorporated into cells was confirmed at regular intervals. The endonuclease was treated with 0.2 mM ZnSO₄ (Sigma) to terminate its enzyme activity. The DNA incorporated into cells was recovered in a cell lysate and run on 20% native-PAGE in the TBE buffer solution. The gel was dried and autoradiographed to confirm the processing of DNA within cells and the end product obtained by the endonuclease reaction.

The EC1 DNA fragment labeled with ³²P was incorporated into IM9 cell line. The DNA incorporated into cells were extracted from cell lysates and 20% native-PAGE was carried out. The processed DNA labeled with ³²P was extracted from the gel and